Miltenyi Biotec

autoMACS® Pro Separator

Short instructions

Version 3

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Version 3

Content

1 Considerations before you start

WARNING! Please read the user manual of this instrument carefully and pay attention to all warnings and precautions.

1.1 autoMACS® Pro cell separation and wash programs

There are two basic strategies for separating specific cell populations: positive selection and depletion. During positive selection, the target cells are magnetically labeled and collected as the positive fraction. During depletion, the unwanted cells are labeled and depleted from the target cells. The target cells are collected as the negative fraction. Furthermore, sequential sorting allows the performance of two consecutive separations. For more information please refer to **www.macscellseparation.com**

The autoMACS® Pro Separator provides a selection of twelve pre-set separation programs. The appropriate program is generally chosen depending on the separation strategy, the target cell frequency, and the level of antigen expression. A decision tree on what cell separation program to choose is located in section 8 of this document. For further information, please refer to the respective Cell Separation Reagent data sheet.

Positive selection programs:
Possel – Positive selection in standard mode:
- for the isolation of cells with frequencies higher than 5% and normal antigen expression.

Possel_s – Positive selection in sensitive mode:
- for the isolation of cells with frequencies higher than 5% and low antigen expression.
- for the isolation of cells with frequencies higher than 5% and normal antigen expression, if recovery is the highest priority.

Posseld – Positive selection in standard mode I, double-column program:
- for the isolation of cells with frequencies lower than 5% and normal antigen expression, in a small elution volume.

Posseld2 – Positive selection in standard mode II, double-column program:
- for the isolation of cells with frequencies lower than 5% and normal antigen expression, if purity is the highest priority.

Note: When using the program **Posselwb**, the whole blood sample will be diluted 3-fold the starting volume.

Posselds – Positive selection in sensitive mode, double-column program:
- for the isolation of cells with frequencies lower than 5% and low antigen expression.

Posselwb – Special positive selection in special mode, double-column program:
- for the isolation of cell subsets from whole blood; cell samples are automatically diluted with Running Buffer.

Depletion programs:

Deplete – Depletion in standard mode:
- for removal of cells with normal antigen expression, if recovery is the highest priority.
- for untouched isolation with MACS® Cell Isolation Kits.

Depletes – Depletion in sensitive mode I:
- for removal of cells with normal antigen expression, if purity is the highest priority.
- for removal of cells with low antigen expression.
- for untouched isolation with MACS Cell Isolation Kits, if purity is highest priority.

Depl05 – Depletion in sensitive mode II:
- for removal of cells with low antigen expression, special program for very sensitive depletion.

Depl025 – Depletion in sensitive mode III:
- for removal of cells with low antigen expression, special program for very sensitive depletion.

A_Depl07 – Depletion in standard mode *via* loading of sample in separate 1 mL stages:
- for removal of cells with normal antigen expression, if recovery is the highest priority. This special program is disabled by default. To enable **A_Depl07**, select **Option**, **User settings**, and **O_progs.**

A_Depls7 – Depletion in sensitive mode *via* loading of sample in separate 1 mL stages:
- for removal of cells with low antigen expression, if purity is the highest priority. This special program is disabled by default. To enable **A_Depls7**, select **Option**, **User settings**, and **O_progs**.

The autoMACS Pro Separator is equipped with reusable autoMACS Columns. After each cell separation, a thorough washing procedure rinses the columns of the autoMACS Pro Separator.

Please find below a list of the available obligatory and optional wash programs for daily use.

Qrinse : Standard short wash program that uses Running Buffer.

Rinse : Extensive rinsing program that uses Washing Solution and Running Buffer.

Clean : Optional, very extensive rinsing program that uses storage solution, Washing Solution, and Running Buffer.

Sleep : It is mandatory to use **Sleep** as the last wash program before overnight storage. This program uses Washing Solution and storage solution. Upon completion of the **Sleep** program, the fluidic system contains 70% ethanol.

1.2 Choose appropriate tube rack

Select the tube rack according to the desired number of samples, number of cells, and sample volume (refer to table 4.1) and ensure that it is pre-cooled to 4°C.

1.3 Prepare cell samples

- Prepare a single-cell suspension and avoid cell aggregates, e.g., using Pre-Separation Filters, 30 µm (# 130-041-407) or Pre-Separation Filters, 70 µm (# 130-095-823).
- Remove dead cells, e.g., using the Dead Cell Removal Kit (# 130-090-101).

2 Setting up and priming the autoMACS® Pro Separator

Note: The connectors for the fluid bottles are color-coded: blue for Running Buffer, green for Washing Solution, black for storage solution, and red for the waste bottle.

2.1 Setup of the instrument

1 Check that all bottles are filled with the appropriate solutions and connected to the appropriate sensor cables. Empty the waste bottle.

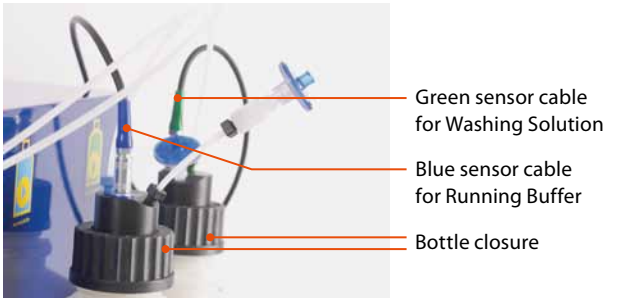


Figure 2.1: Bottle closures and sensor cables of the fluid bottles.

2 Check that the MACS MiniSampler, the 2D code reader, and the fluid sensor cables are attached correctly to the back of the instrument.

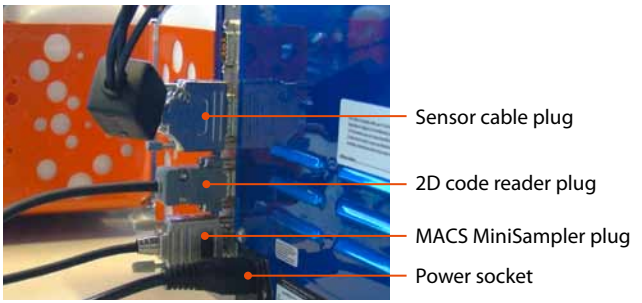


Figure 2.2: Plugs of accessories attached to the back of the instrument.

3 Switch ON the autoMACS® Pro Separator. After initialization of the instrument, the touchscreen displays the **Status** menu.

Figure 2.3: Location of the ON/OFF switch.

2.2 Priming of the instrument

To prime the instrument, go to the **Separation** menu.

1 Select the **Separation** menu.

2 Select **Wash Now** from the lower navigation bar.

3 Select **Rinse** and **Run**.

2.3 Monitoring the instrument status prior to cell separation

The instrument status can be determined by viewing the **Status** menu at any time.

1 Sample status
2 Fluid bottle filling status
3 Column status
4 Tube rack status
5 MACS MiniSampler status

Figure 2.4: Overview of the “Status“ menu.

2.3.1 Status of fluid bottles

Confirm that the fluid bottle status is ready.

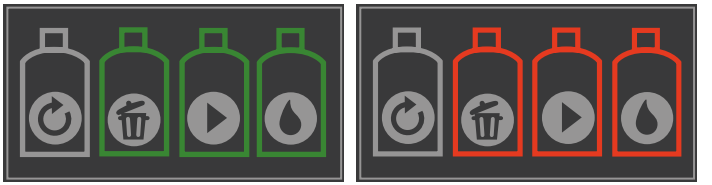


Figure 2.5: Left: Fluid bottles are shown in green indicating ready.
Right: Fluid bottles are shown in red and need to be replaced.

Bottle	Symbol	Symbol color and user action
Running Buffer		Green: no action required Red: refill bottle Gray: connect bottle sensor
Washing Solution		Green: no action required Red: refill bottle Gray: connect bottle sensor
Storage solution		Gray: no liquid detection; visually check volume
Waste		Green: no action required Red: empty waste or wrong sensor cable is connected Gray: connect bottle sensor

Table 2.1: Status of fluid bottles displayed in the "Status" menu. The color of the bottle symbols indicates the color code of the sensor cables. The filling status of the storage solution cannot be detected, since it does not contain electrolytes.

2.3.2 Status of columns

Confirm that the column status is ready.

Green: no action required
Red: exchange column
Grey: no column has been installed

2.3.3 Status of MACS MiniSampler

Confirm that the MACS MiniSampler is correctly installed.

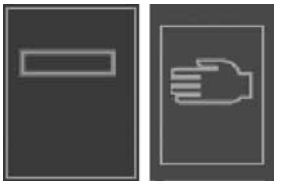


Figure 2.6: MACS MiniSampler status graphic. Left: The MACS MiniSampler was successfully installed. Right: No MACS MiniSampler was detected.

The instrument is now ready for use.

3 Preparation of samples

It is recommended to use single-cell suspension for cell separation, devoid of cell aggregates and dead cells. Typically, 1×10^7 cells are resuspended in 80 µL of buffer and labeled with 20 µL of MicroBeads, leading to a total labeling volume of 100 µL. When working with higher cell numbers, scale-up all reagent volumes and total volumes accordingly. When working with fewer than 1×10^7 cells, do NOT scale down the volumes, but use the same volumes as indicated.

In the table below, the dilution volumes account for the first step of labeling. For manual labeling, please refer to the respective Cell Separation Reagent data sheet for ongoing procedures. Minimal and maximal volumes and total cell numbers in table 4.1 account for autolabeling samples only. For a current list of Cell Separation Reagents and Kits that are optimized for cell separations with the autoMACS Pro Separator autolabeling feature, please contact Mitenyi Biotec Technical Support.

Cell Separation Reagent	Strategy	Number of Reagents	Dilution volume	autolabeling			
				Minimal volume*	Minimal total cell number	Maximal volume	Maximal total cell number
Chill 5 Rack[1]							
Direct MicroBeads human, rat, non-human primate	Positive selection or depletion	1	10^7 cells per 80 µL	160 µL	2×10^7	1600 µL	2×10^8
Direct MicroBeads, mouse	Positive selection or depletion	1	10^7 cells per 90 µL	180 µL	2×10^7	1800 µL	2×10^8
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	0.25 mL		1 mL	
Cell Isolation Kits	Untouched isolation	2	10^7 cells per 40 µL	160 µL	4×10^7	1600 µL	4×10^8
Cell Isolation Kits	Untouched isolation	3	10^7 cells per 30 µL	120 µL	4×10^7	1200 µL	4×10^8
MicroBead Kits	Positive selection or depletion	2	10^7 cells per 60 µL	120 µL	2×10^7	1200 µL	2×10^8
Chill 15 Rack[2]							
Direct MicroBeads human, rat, non-human primate	Positive selection or depletion	1	10^7 cells per 80 µL	160 µL	2×10^7	5200 µL	6.5×10^8
Direct MicroBeads, mouse	Positive selection or depletion	1	10^7 cells per 90 µL	180 µL	2×10^7	5850 µL	6.5×10^8
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	1 mL		4 mL	
Cell Isolation Kits	Untouched isolation	2	10^7 cells per 40 µL	160 µL	4×10^7	5200 µL	1.3×10^9
Cell Isolation Kits	Untouched isolation	3	10^7 cells per 30 µL	120 µL	4×10^7	5850 µL	1.3×10^9
MicroBead Kits	Positive selection or depletion	2	10^7 cells per 60 µL	120 µL	2×10^7	5850 µL	6.5×10^8
Chill 50 Rack[3]							
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	4 mL		8 mL	

[1] Max. number of samples: 6; min. first incubation volume: 0,2 mL; max. final labeling volume: 2 mL.
[2] Max. number of samples: 5; min. first incubation volume: 0,2 mL; max. final labeling volume: 6,5 mL.
[3] Max. number of samples: 3; min. first incubation volume: 4 mL; max. final labeling volume: 8 mL.
* When working with fewer cells than the necessary minimal volume, resuspend cells in the stipulated minimal volume.

Table 3.1: Dilution volumes for the first labeling step and MACS Chill Rack specifications for autolabeling, including minimal and maximal volumes and cell numbers.

4 Select the appropriate tube rack

MACS Chill Racks are automatically detected by the autoMACS Pro Separator. Three different tube racks are available for processing sample volumes between 0.2 mL and 50 mL.

1 Select the appropriate tube rack according to table 4.1.

2 Cool down the tube rack for 3–4 hours in a refrigerator (2–8 °C) or until the coolant becomes solid.
Do not cool below 0 °C as samples may freeze.

3 Equip the tube rack with sample tubes and fraction collection tubes.

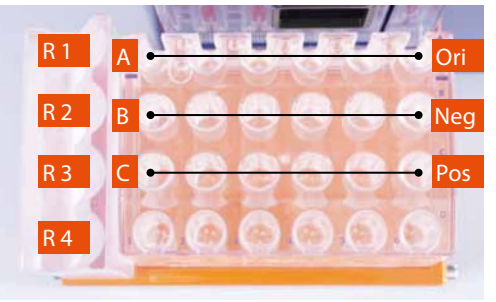


Figure 4.1: Specifications of a chill rack, shown here a Chill 5 Rack and MACS Reagent Rack 4. R1–R4 correspond to the reagent rack positions. Row A contains the tubes for original samples, i.e., "Ori". Row B contains tubes for negative, unlabeled fractions, i.e., "Neg". Row C contains tubes for the positive, magnetically labeled fractions, i.e., "Pos.".

Rack type and symbol	Slots	Maximal number of samples	Manual labeling	Autolabeling	
			Maximal sample volume	Minimal first incubation volume	Maximal final labeling volume
Chill 5 	24×5 mL	6 (5 mL tubes)	2.5 mL	0.2 mL 0.25 mL*	2.0 mL 1 mL*
Chill 15 	15×15 mL 5×5 mL	5 (15 mL tubes)	12.5 mL	0.2 mL 1 mL*	6.5 mL 4 mL*
Chill 50 	6×50 mL 3×15 mL 3×5 mL	3 (50 mL tubes)	50 mL	4 mL*	8 mL*

* Volumes refer to whole blood samles, only.

Table 4.1: MACS Chill Rack specifications for manual labeling and autolabeling. For further details on sample volumes for autolabeling, refer to table 3.1.

5 Labeling

Cells can be labeled with MACS MicroBeads either manually or using the autolabeling function of the autoMACS Pro Separator. For detailed information on manual labeling, please refer to the Cell Separation Reagent data sheet. For a list of Cell Separation Reagents optimized for autolabeling, please contact Technical Support.

For autolabeling, insert the MACS Reagent Rack 4 onto the MACS MiniSampler.

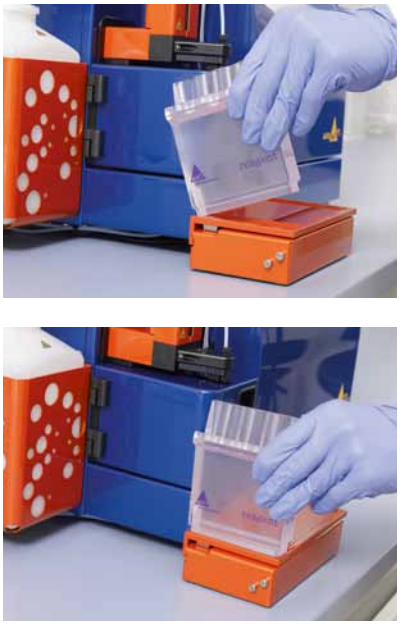


Figure 5.1: The MACS Reagent Rack 4 snaps into position as illustrated above.

Entry of reagents for autolabeling using the 2D code reader

1 Go to the **Reagent** menu and highlight the position where the vial will be placed in the reagent rack. Four positions are available: **R1**, **R2**, **R3**, and **R4**.

Figure 5.2: The rack position "R1" was selected for reagent assignment.

2 Select **Read Reagent** to activate the 2D code reader.

3 Present a reagent vial in front of the 2D code reader. Ensure that the 2D code is facing the blinking code reader–light.

Figure 5.3: The optimal reading distance is 0.5–2.5 cm from the code reader cover, tilt the vial as shown above.

4 After successfully scanning a reagent vial, the next available reagent rack position will be automatically highlighted.

5 To view details on a scanned Cell Separation Reagent, highlight the respective rack position.

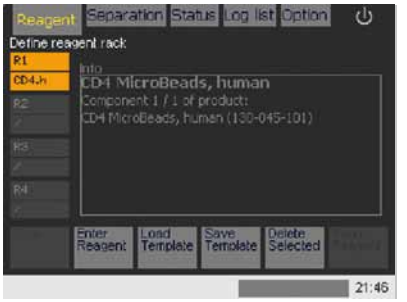


Figure 5.4: Details on the Cell Separation Reagent assigned to rack position "R1" are displayed.

6 Insert the reagent vial into the appropriate rack position.

Manual entry of reagents for autolabeling

This option is only recommended, if the reagent cannot be identified by the 2D code reader.

1 Go to the **Reagent** menu and highlight the position where the vial will be placed in the reagent rack. Four positions are available: **R1**, **R2**, **R3**, and **R4**.

Figure 5.5: The rack position "R1" was selected for reagent assignment.

2 Select **Enter Reagent** from the lower navigation bar. Enter the reagent specific product order number. The product order number is located on the Cell Separation Reagent data sheet. If this is not available, visit the respective product page at www.miltenyibiotec.com to download a printable PDF of the document.

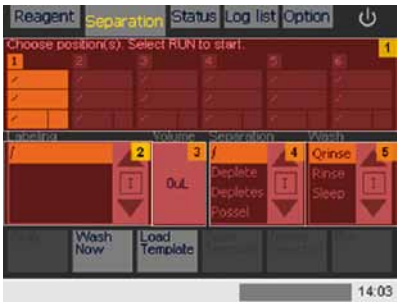
3 If a correct product number was entered, the software will immediately recognize and list the reagent or kit components. To confirm your choice, select the listed reagent by using the touch screen (☑). The next available rack position will be automatically highlighted. Repeat the procedure for the remaining reagents or kit components.

Figure 5.6: Entry of the components of the NK Cell Isolation Kit II, mouse . The reagent NK Cell Biotin-Antibody Cocktail, mouse was assigned to rack position "R1". The reagent Anti-Biotin MicroBeads was assigned to rack position "R2".

4 Select **Ok** to complete the reagent entry.

6 Cell separation

Place the appropriate Chill Rack onto the MACS MiniSampler. For details refer to table 4.1. Go to the **Separation** menu to set up the sample rack template.



1 Sample rack template
2 Sample labeling options
3 Sample processing volume
4 Separation program
5 Wash procedure

Figure 6.1: Sample rack template in the “Separation” menu.

6.1 Cell separation after autolabeling

1 Highlight the desired position(s) in the sample separation template.

2 Assign an autolabeling program from the **Labeling** submenu to the respective position.

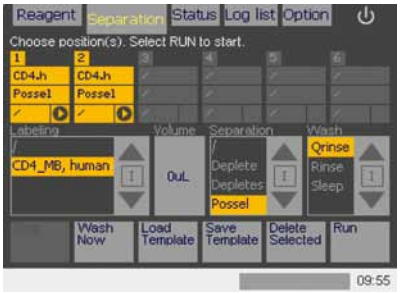


Figure 6.2: The Cell Separation Reagent CD4 MicroBeads, human was assigned to rack positions 1 and 2. The separation program “Possel” and wash program “Qrinse” were automatically selected.

3 (Optional) The recommended cell separation and wash program will be automatically displayed after choosing the autolabeling program. It is possible to change the separation program or the wash program between samples or to assign the **Sleep** program after finishing the last sample. Highlight the desired cell separation and wash program in the **Separation** and **Wash** submenus, respectively.

4 Insert a sample volume in the **Volume** submenu using the numeric keypad. Select **Enter**. For detailed information on sample volumes, refer to table 3.1.

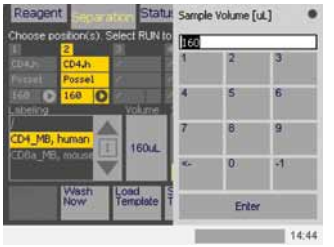


Figure 6.3: A sample volume of 160 µL was entered in the "Volume" submenu.

5 Select **Run** to start the cell separation. Select **Ok** to confirm that enough buffer is available for processing all samples.

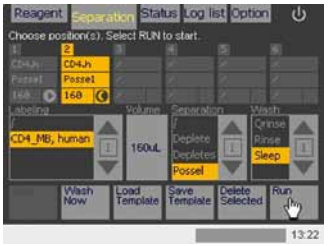


Figure 6.4: A cell separation using CD4 MicroBeads, human will be performed on sample positions 1 and 2. After processing sample 2 the "Sleep" program will be performed as a final wash step before the instrument goes into sleep mode. Clicking "Run" will start the experiment.

6.2 Cell separation after manual labeling

1 Highlight the desired position(s) in the sample separation template.

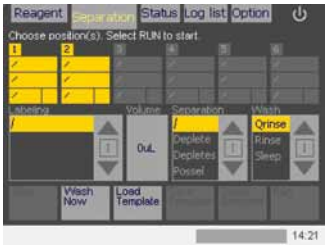


Figure 6.5: Select multiple sample positions in order to set them up simultaneously.

2 Select "/" from the **Labeling** submenu for manual labeling.

3 (Optional) It is not mandatory to assign a volume to manually labeled samples. However, the autoMACS Pro Separator requires this information to calculate and display the total sample processing time. Unless otherwise indicated it is recommended to dilute manually labeled samples to a volume of 500 µL per 10^8 cells. For detailed information, please refer to the corresponding data sheet. Enter the sample volume in the **Volume** submenu using the numeric keypad. Select **Enter**.

Note: If the **Clean** program has been enabled, it will also appear in the **Wash** submenu.

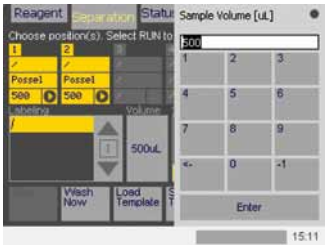


Figure 6.6: A volume of 500 µL was assigned to samples 1 and 2.

4 Assign a separation program and a washing program to each sample position. The selected programs will be displayed in the programming field. For details refer to section 1.1.

5 Select **Run** to start the cell separation. Select **Ok** to confirm that enough buffer is available for processing all samples.

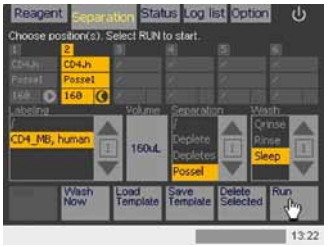


Figure 6.7: The separation program “Possel” will be performed on sample positions 1 and 2. After processing sample 2 the “Sleep” program will be performed as a final wash step before the instrument goes into sleep mode. Clicking “Run“ will start the experiment.

6.3 Monitoring the cell separation process

Use the **Status** menu display to view the overall instrument status. For more details, please refer to section 2.3.

6.3.1 Sample processing status

Sample processing statuses are displayed as color-coded graphics.

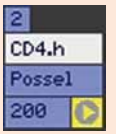
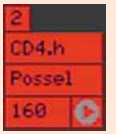
	Graphic	Definition
1		Status: Waiting. Sample processing has not yet started.
2		Sample autolabeling is underway.
3		Incubation of cells with labeling reagents.
4		Sample is being processed, e.g., sample uptake.
5		Rinsing
6		Sample processing is completed.
7		Progress has been stopped or cancelled.

Table 6.1: Sample processing statuses displayed in the “Status” menu. Presented here is a cell separation with CD4 MicroBeads, human using the autolabeling feature.

6.3.2 Fluid bottle illuminations

The autoMACS Pro Separator provides a fluid bottle illumination that facilitates monitoring of the instrument's status, even from across the laboratory.

Code	Status	User action
Green	Ready for separation	No action required.
Blue	Instrument operating	No action required.
Yellow	Not ready for separation	Run wash program (**Rinse** or **Qrinse**) before starting a separation.
Red	Error	Check screen for error detection.
Purple	Program **Sleep** is completed	Switch OFF autoMACS Pro Separator.
Blinking	Action required	Check screen for required action.

Table 6.2: Fluid bottle illumination.

6.4 Setting the autoMACS Pro Separator in sleep mode

Note: For daily usage the instrument should not be switched OFF but placed into sleep mode.

1 ⏻ Press the shutdown symbol (upper right-hand corner of the display).

2 Alternatively, select **Sleep** program as the last washing step.

6.5 (Optional) Switch OFF the instrument for long-term storage

Note: To store the autoMACS Pro Separator for a period longer than two weeks run the **Store** program.

1 Go to the **Option** menu and select **Special**.

2 Select **Store** and press **Run**.

3 Replace the columns with column substitutes (refer to section 3.3.4 of the user manual).

4 Select **Done**.

5 Switch OFF the autoMACS Pro Separator using the main power switch.

7 Maintenance

7.1 Rinsing programs

Program	Description	Recommended usage	Duration
Qrinse	Standard short rinse of separation columns and tubing system with Running Buffer	Between separations of frequent cells (>5 %)	1.5 min
Rinse	Rinse of separation columns and tubing system with Washing Solution and Running Buffer	Between and before separations of rare cells (<5%)	4 min

Table 7.1: Specifications of the rinsing programs.

7.2 Daily maintenance programs

Program	Description	Recommended usage	Duration
Rinse	Rinse of separation columns and tubing system with Washing Solution and Running Buffer	Prior to first separation	4 min
Clean	Rinse of separation columns and tubing system with storage solution, Washing Solution, and Running Buffer	After whole blood and bone marrow applications	7 min
Sleep	Rinse with Washing Solution followed by filling with storage solution	Before switching OFF the autoMACS Pro Separator	5 min

Table 7.2: Specifications of programs for daily maintenance.

7.3 Periodic maintenance

Action	Description	Recommended usage	Duration
Column exchange using the **Col_ex** program	Replacement of separation columns	Every two weeks OR after 100 separations, whichever comes first	6 min
Running the **Safe** program	Decontamination procedure with MACS Bleach Solution	Every 3–6 months	21 min
Cleaning the pump syringe	Cleaning of pump syringe (refer to user manual)	Every 1–3 months	
Running the **Store** program	Rinse with Washing Solution, followed by storage solution; replacement of columns with substitutes	Before storing the instrument for a period longer than two weeks	

Table 7.3: Specifications of programs for periodic maintenance.

7.4 Column exchange

Replace autoMACS Columns every two weeks or after 100 separations, whichever comes first.

1 Go to the **Option** menu. Select **Special** and **Col_ex**.

2 Press **Run**.

3 When prompted, exchange the columns.

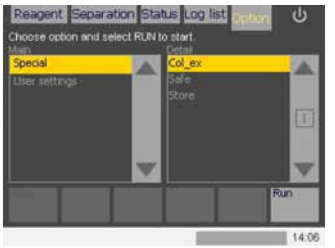


Figure 7.1: "Col_ex" program.

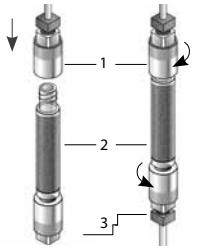
4 Open front door and note the positions of the columns (column 1: left; column 2: right). Exchange one column at a time.

5 Remove column from slot; unscrew top column connector followed by the bottom column connector as shown in figure 7.2.

6 Dispose of the expired column.

7 Point the bottom of the fresh column towards the autoMACS Pro Separator.

8 Insert bottom column connector. Screw in the column by turning it clockwise. Repeat the procedure for the top column connector.



1 Top Column Connector
2 autoMACS Column
3 Bottom Column Connector

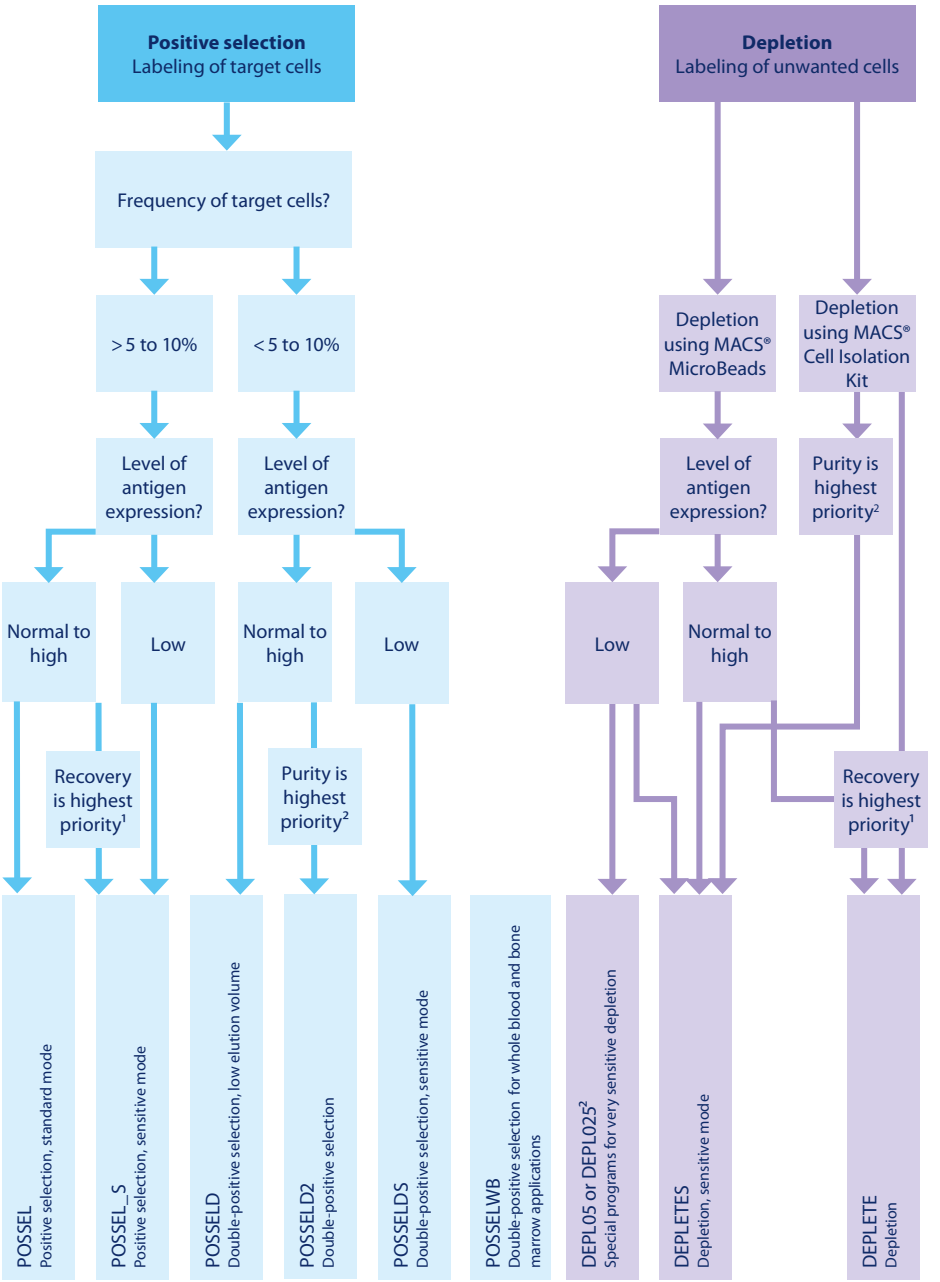
Figure 7.2: Top: Exchange of the column. Bottom: Starting the “Col_ex” program.

9 Push column into the magnet housing, with the top column connector sitting on the guide in the column slot.

10 Repeat installation for the second autoMACS Column.

11 After exchange of separation columns, select **Done**. The autoMACS Pro Separator system will be automatically primed with Running Buffer and is then ready for cell separation.

8 Decision tree for the optimal separation program



[1]Purity will slightly decrease
[2]Recovery will slightly decrease

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